Organ specificity in the circadian control of plant gene expression

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Abstract

Of the many plant genes whose expressions are controlled by the circadian clock, one of the phosphoenolpyruvate carboxylase kinase genes in soya bean (glycine max) exhibits the unusual property that its control is organ-specific – it is under circadian control in leaves but not in roots. Preliminary experiments suggest that the same is true for at least one gene in Arabidopsis thaliana. It will be important to define the extent and function of this phenomenon and the underlying mechanism.

Introduction

Many aspects of plant behaviour exhibit circadian rhythmicity, including several facets of carbon and nitrogen metabolism [1–3]. Microarray experiments have suggested that some 6% of Arabidopsis genes are expressed rhythmically and have identified ‘clusters’ of metabolically related genes that are expressed at particular times of the day [2]. For example, several genes expressed in late subjective afternoon encode proteins that function in the storage, transport or utilization of sugars. The promoters of many genes expressed at this phase contain an ‘evening element’ that is responsible for this timing [2].

Individual plant cells contain separate, uncoupled copies of the ‘central clock’ [4], with cell-specific modification of the components allowing generation of rhythms with different periods. It is generally assumed that, if a plant gene is controlled by the clock in one cell type, it will be so controlled in all cells in which it is expressed. However, it is a characteristic of metabolic pathways that their timing and co-ordination depend crucially on the organs or cell types involved. Hence circadian control of metabolic gene expression might be organ-specific. Our data suggest that this is the case in soya bean (Glycine max) and we are addressing this question systematically using Arabidopsis thaliana.

Control of PPCK gene expression

Phosphoenolpyruvate carboxylase (PEPc) plays many roles in plant metabolism, including the primary fixation of CO2 at night in CAM (crassulacean acid metabolism) plants, the production of malate in legume root nodules and provision of precursors for biosynthesis through its anaplerotic role in almost all cell types. Flux through PEPc is largely controlled by expression of PPCK genes encoding PEPc kinase, which phosphorylates and activates PEPc [5]. Unlike other protein kinases, PEPc kinase simply comprises a kinase catalytic domain with no regulatory regions, and it is controlled mainly by expression.

CAM plants show persistent circadian rhythms of CO2 metabolism [6,7]. For example, leaves held in constant darkness in CO2-free air show a rhythm of CO2 output that is caused by periodic refixation of respiratory CO2 by PEPc. These periods of CO2 fixation correlate with times at which PEPc is phosphorylated owing to circadian expression of PPCK [8]. It seems likely that this circadian expression of PPCK is an indirect effect mediated by metabolites rather than the result of a direct connection between the ‘central clock’ and PPCK expression [7]. Arabidopsis contains two PPCK genes, but several experiments using both RT–PCR and promoter–luciferase fusions have failed to provide any evidence for circadian control of either gene. Hence, until recently, there was no evidence that PPCK expression is controlled circadianly other than in CAM species.

Soya bean PPCK4 is under circadian control in leaves but not in roots

Soya bean contains at least four PPCK genes [9]. In studies of their spatial and temporal patterns of expression, we found no evidence for circadian control of GmPPCK1–3. However, the promoter of GmPPCK4 contains the core ‘evening element’ and this gene is under robust circadian control in constant light in leaves but not in roots [9]. In leaves, it is expressed in late subjective day. Expression of GmPPCK4 in leaves also cycles in constant darkness, but under these conditions the rhythm dies out quickly, as for many other rhythms of plant gene expression. We used GmpLHY, the putative soya bean orthologue of the A. thaliana clock gene LHY, as a marker for operation of the central clock. As expected, it is expressed in early subjective day. Thus the clock runs in both leaves and roots but is only connected to GmPPCK4 expression in leaves [9].

The reduction of nitrate to ammonium ions followed by their incorporation into amino acids requires energy
and reducing power. Hence a plausible explanation for the circadian control of GmPPCK4 in leaves is that it directs carbon towards biosynthesis, particularly of amino acids, via activation of PEPc during the latter part of the day. While the ‘function’ of GmPPCK4 in roots might be similar, the ‘timing’ of its expression is not likely to be as critical as in leaves.

**Organ specificity of circadian control in Arabidopsis**

Harmer et al. [2] identified several clock-controlled Arabidopsis genes that are expressed in whole seedlings in late subjective afternoon and are involved in sugar metabolism. They suggested that the circadian clock might help to allocate sugar carbon to different pathways. In preliminary experiments we have studied one of these genes, At2g18700 (involved in trehalose metabolism). The data suggest that transcripts from this gene may cycle in leaves but not in roots. However more detailed work using microarrays is needed both to confirm our preliminary observations and to define the extent of this behaviour in Arabidopsis.

**Conclusions**

Our work on soya bean PPCK4 has shown for the first time in plants that circadian control of gene expression can be organ-specific. Given the significant number of genes that are under circadian control, it is necessary to identify the importance of this effect and the underlying mechanism.

Although it has yet to be shown that the cycles in GmPPCK4 and At2g18700 translate into cycles of activity, it seems likely that the role of circadian control lies in the co-ordination of metabolism. Several studies in mammals suggest that there are reciprocal links between metabolism and the central clock, and that clock control is organ-specific [10,11]. One potential mechanism for the organ specificity of circadian control in plants is that metabolites are involved in the output pathway cycle in leaves but not in roots. A second possibility is organ-specific expression of a protein that is required to couple the central clock to outputs, such as ZGT (abbreviated from the Chinese phrase zhong guang tiaokong, meaning clock- and light-controlled) in tobacco. This is a light- and clock-regulated protein of unknown structure that links the clock to rhythmic expression of LHCB genes [12].

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**References**


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